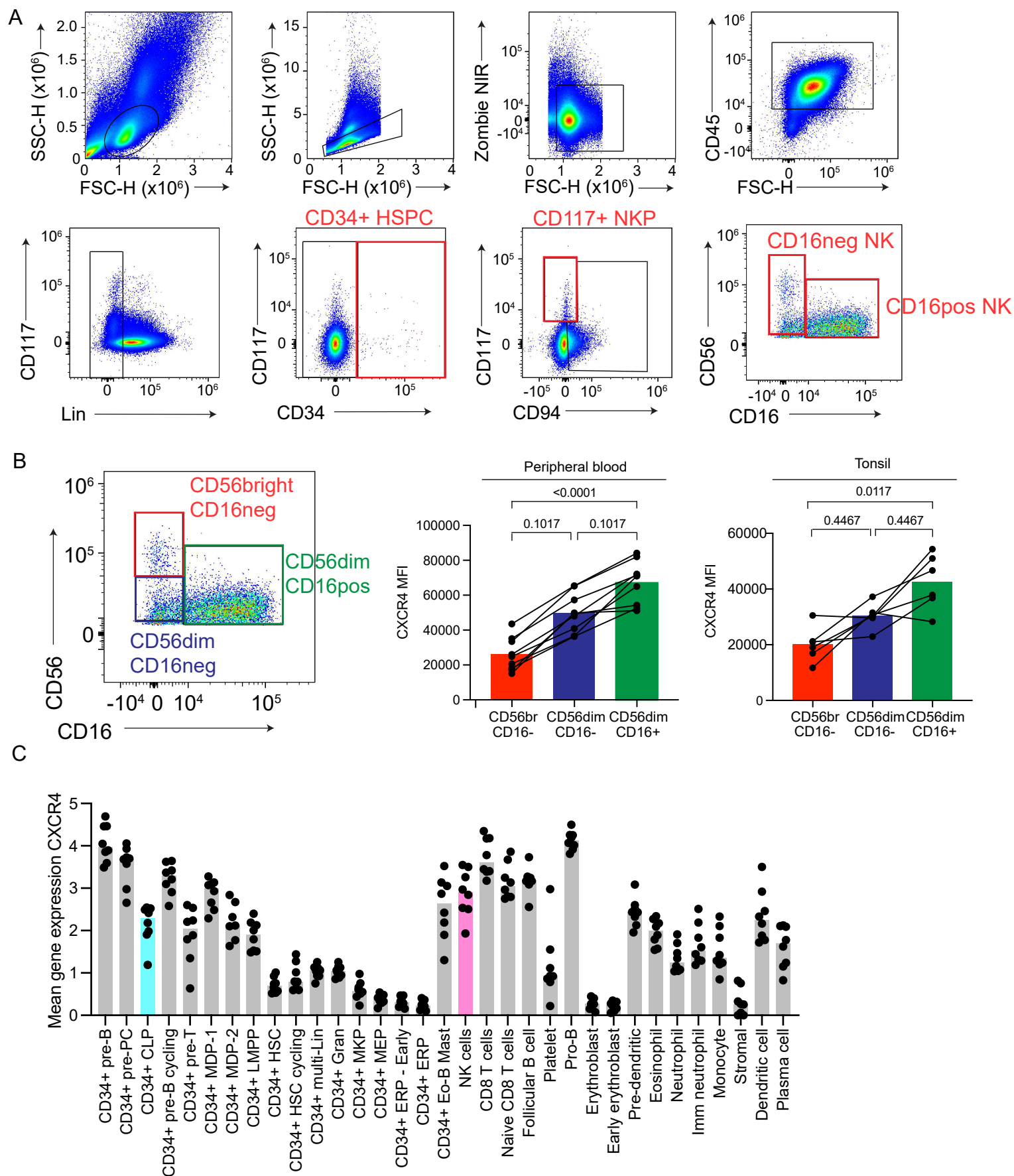
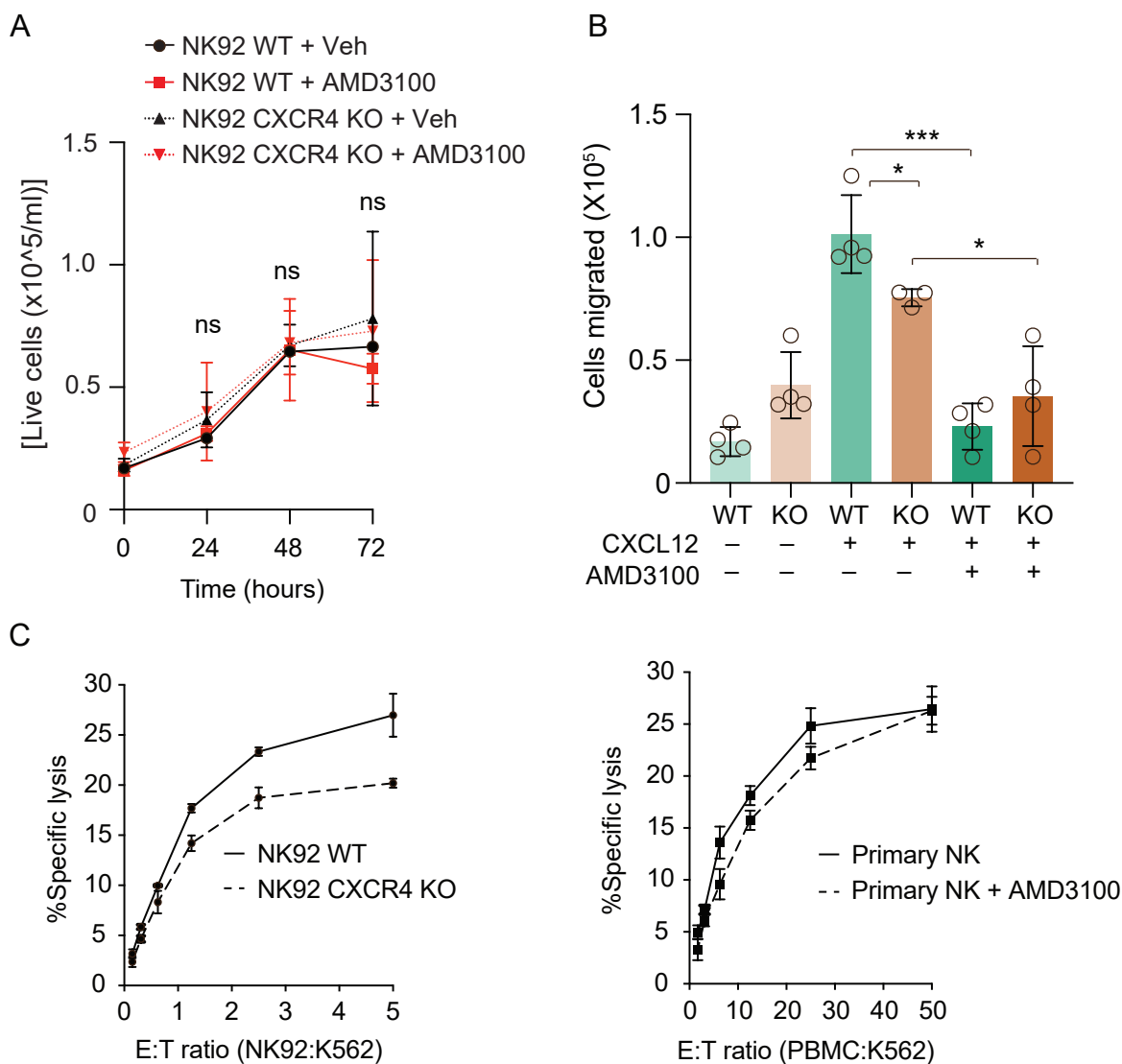


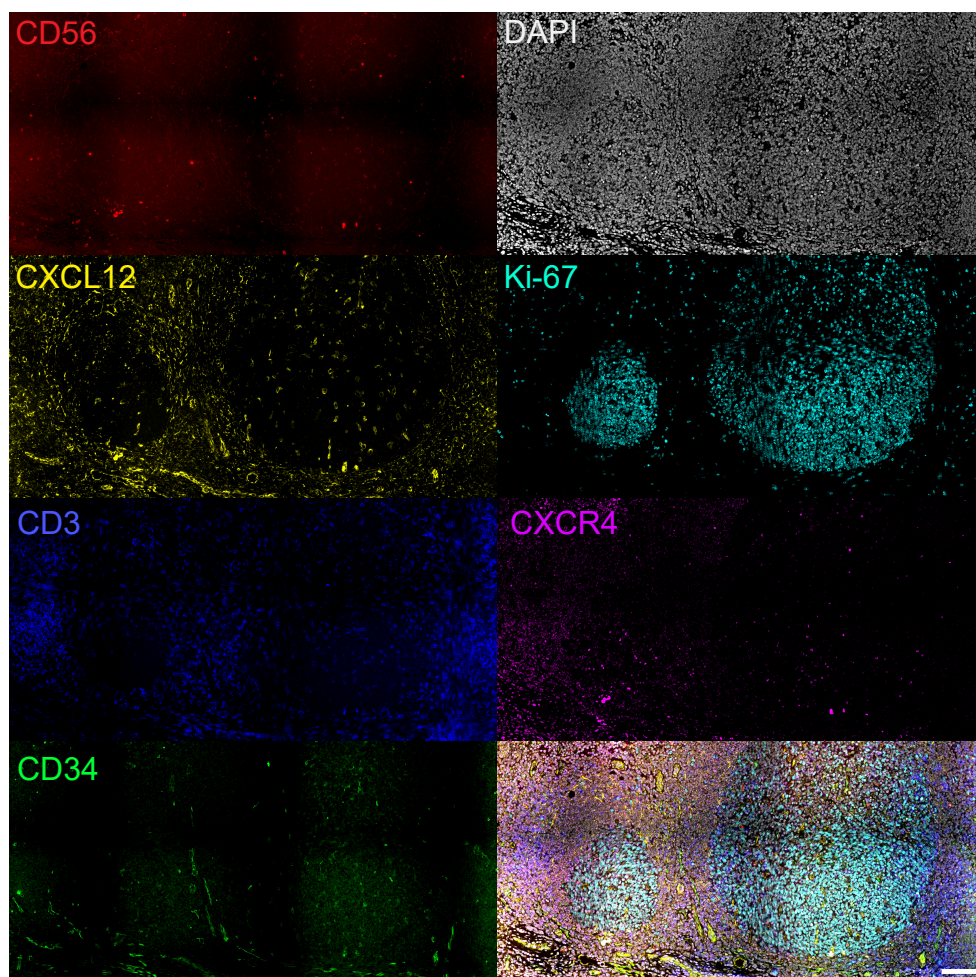


Supplemental Figure 1. Gating strategy and CXCR4 expression. A) Gating strategy used for analysis of NK cell developmental intermediates in peripheral blood and tonsil. B) Additional gating of mature NK cell subsets from peripheral blood and tonsil (left). Quantitation of CXCR4 MFI from peripheral blood and tonsil mature NK cell subsets. $n=9$ (peripheral blood) and 6 (tonsil) donors; p-values generated by Friedman test. C) Mean gene expression of CXCR4 from Hay et al. (ref. 40) highlighting CD34+ common lymphoid progenitors (cyan) and NK cells (pink). Mean counts per 10,000 cells normalized.

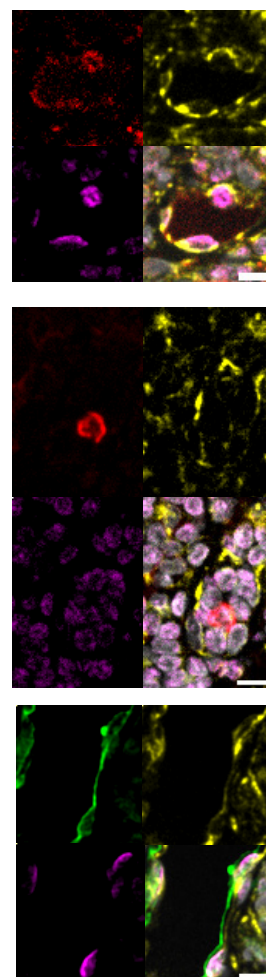


Supplemental Figure 2. Proliferation assays, transwell assays, and killing assays A) NK92 WT and CXCR4 KO cell proliferation measured over 72h in the presence of Vehicle (water) or 10 μM AMD3100. Lines show mean $\pm$ SD (n=3 independent experiments). ns=not significant by two-way ANOVA. B) CXCL12-directed chemotaxis of NK92 WT and CXCR4 KO cells $\pm$ AMD3100 in transwell migration assays; mean $\pm$ SD (n=3 independent experiments). P values determined by Mann-Whitney test between samples as indicated. C) Chromium cytotoxicity assays using NK92 (left) or peripheral blood mononuclear cells (right) co-cultured with K562 target cells at indicated effector: target (E:T) ratios. Mean $\pm$ SEM.

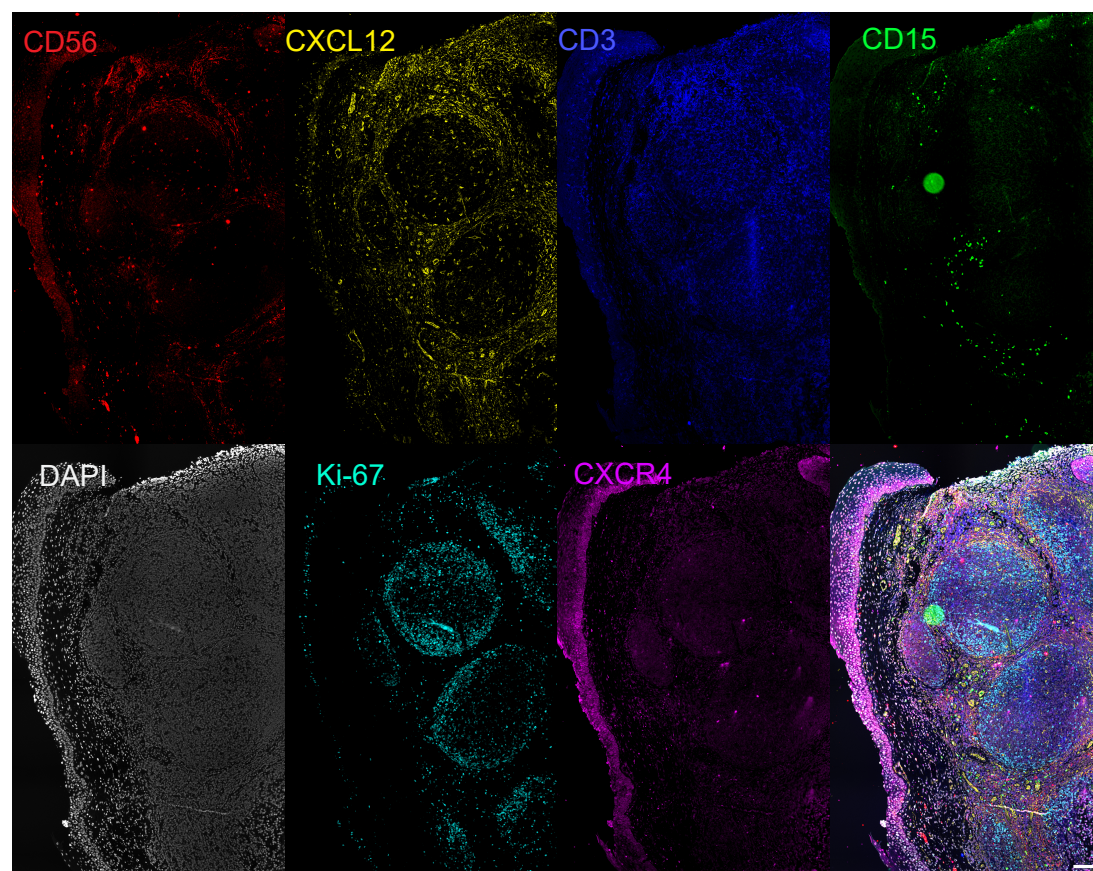
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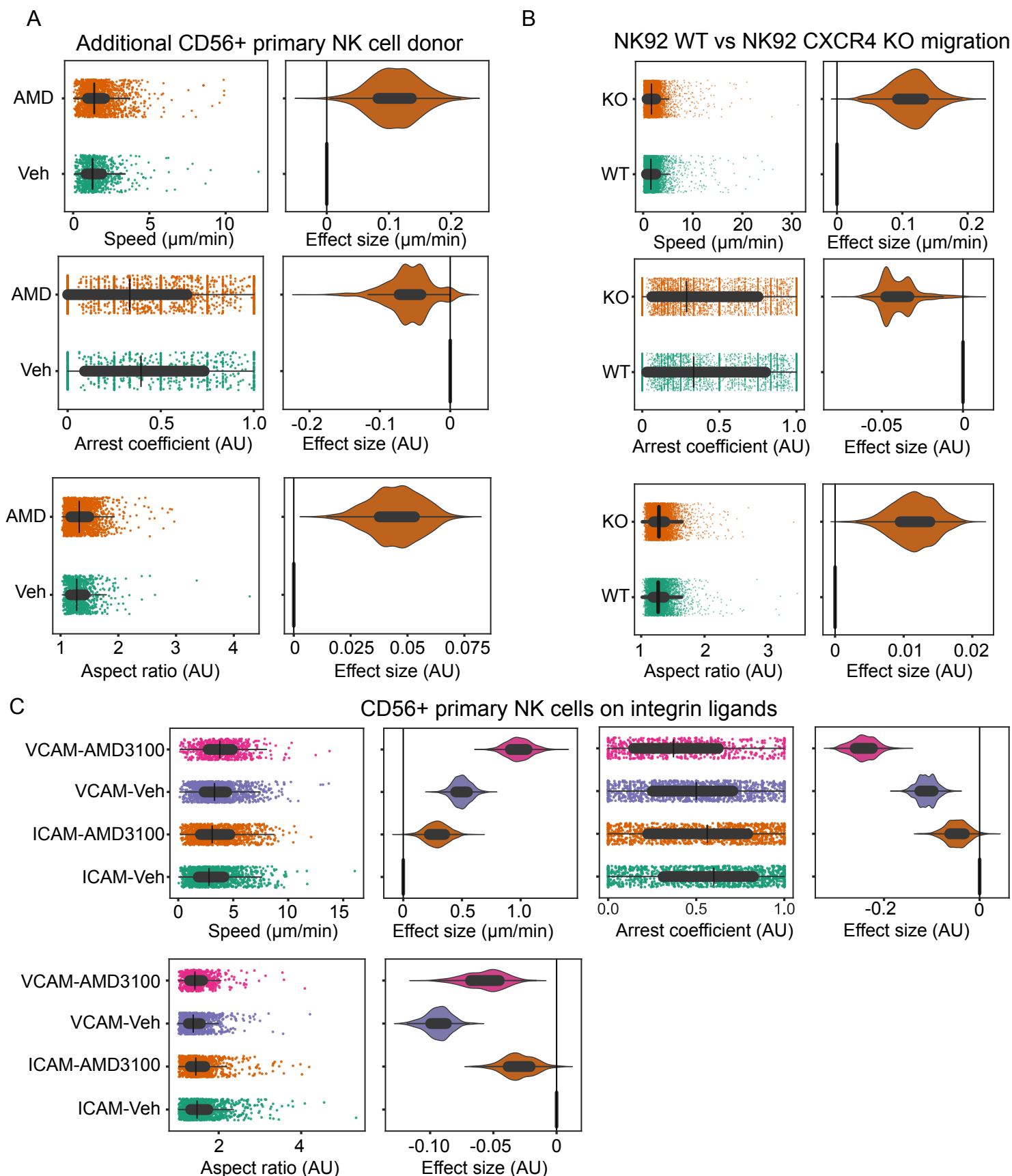
B



C

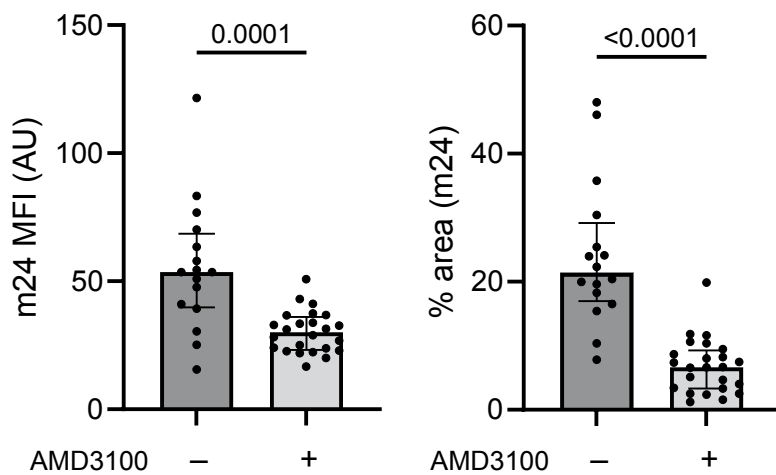


Supplemental Figure 3. Cyclic immunofluorescence (CyCIF) imaging of pediatric tonsil identifying CXCL12-rich stromal regions and associated immune populations. A) Representative CyCIF panel from pediatric tonsil tissue showing individual channels for CD56 (red), DAPI (gray), CXCL12 (yellow), Ki-67 (cyan), CD3 (blue), CXCR4 (magenta), and CD34 (green). B) Insights highlight CXCR4⁺ CD56⁺ and CXCR4⁺ CD34⁺ cells positioned near CXCL12-producing stromal structures. C) Second representative region with individual channels for CD56 (red), DAPI (gray), CXCL12 (yellow), Ki-67 (cyan), CD3 (blue), CXCR4 (magenta), and CD15 (green).

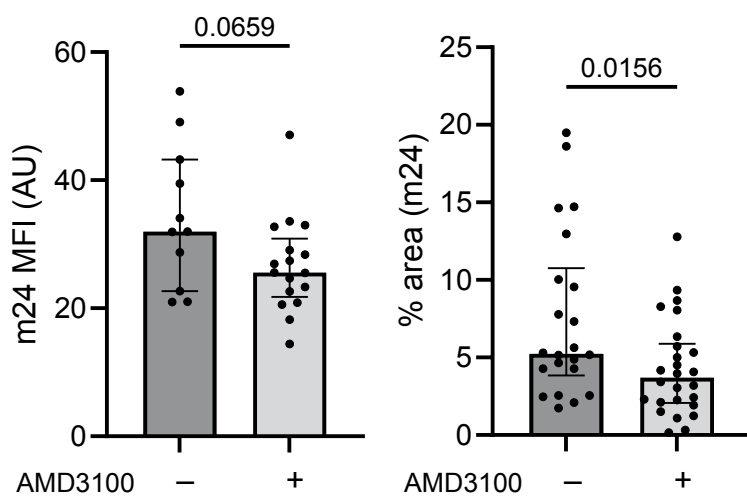


Supplemental Figure 4. Plots of migration and morphological metrics from additional experimental replicates. A) NK cells were isolated from peripheral blood of healthy donors and incubated on EL08.1D2 stromal cells in the presence of AMD3100 or Vehicle as indicated then imaged by confocal microscopy for 4 hours at 2 min intervals. Point plots display pooled single-cell metrics with effect size distributions. B) NK92 WT or CXCR4-KO cells were incubated on EL08.1D2 stromal cells then imaged by confocal microscopy for 4 hours at 2 min intervals. Point plots display pooled single-cell metrics with effect size distributions. C) NK cells were isolated from peripheral blood of healthy donors and incubated on recombinant VCAM1 or ICAM1 in the presence of AMD3100 or Vehicle as indicated then imaged by confocal microscopy for 4 hours at 2 min intervals. Point plots display pooled single-cell metrics with effect size distributions.

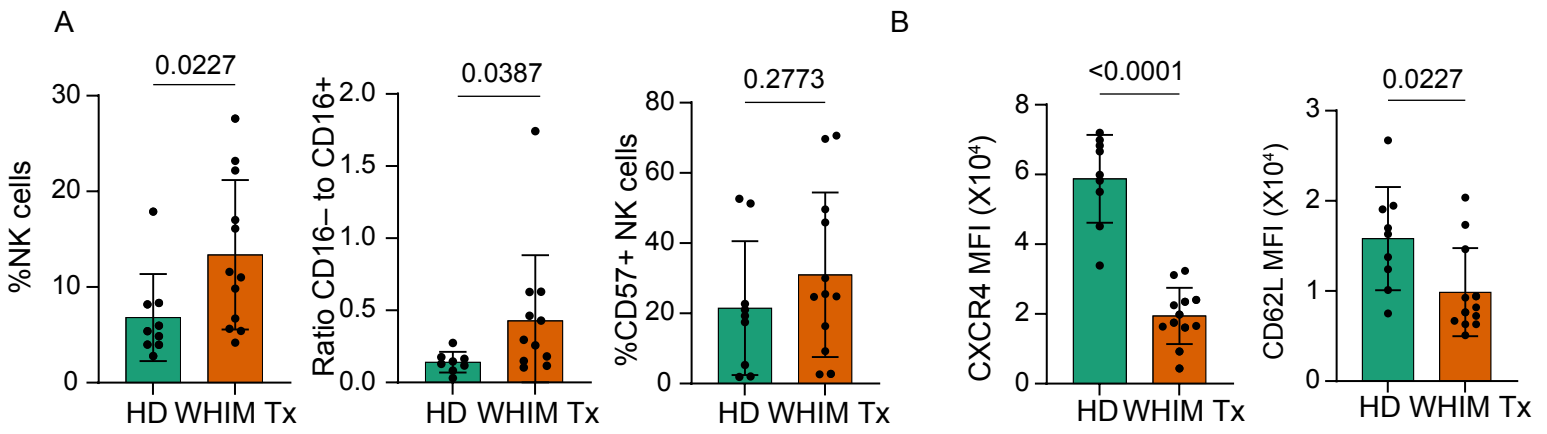
Donor 2



Donor 3



Supplemental Figure 5. Additional donors for m24 MFI and percent area. Quantification of m24 MFI and percent area from two additional healthy donors (see Fig. 4E for Donor 1). Each dot represents a single cell from one donor. Horizontal bars indicate mean ± SD. Statistical comparison was performed using a two-tailed Mann-Whitney test.



Supplemental Figure 6. NK cell numbers from WHIM syndrome patients. A) NK cell frequency, CD16+ and CD57+ subsets in healthy donors (HDs) or WHIM patients treated with filgrastim or plerixafor (WHIM Tx). n=9 HD samples and 12 WHIM patient samples; mean±SD; Mann-Whitney test. B) CXCR4 and CD62L on NK cells from HD and treated WHIM patients (WHIM Tx); mean±SD; Mann-Whitney test.

Supplemental Table 1: Antibody panel used for flow cytometric analysis of NK cell developmental subsets

Antigen	Clone	Fluorochrome	Vendor	Catalog #	Dilution
CD45	HI30	BUV805	BD Biosciences	612891	1:100
CD94	HP-3D9	BUV395	BD Biosciences	743954	1:150
CD3	UCHT1	BUV496	BD Biosciences	612940	1:200
CD14	M5E2	BUV496	BD Biosciences	750381	1:200
CD19	SJ25C1	BUV496	BD Biosciences	612938	1:200
CD56	NCAM1 6.2	BUV563	BD Biosciences	612928	1:100
CD57	QA17A O4	BV510	Biolegend	393314	1:100
CD117	104D2	BV711	Biolegend	313230	1:100
CD34	561	PE Dazzle	Biolegend	343534	1:100
CXCR4	12G5	PE-Cy7	Biolegend	306514	1:100
NKp80	REA845	APC	Miltenyi	130-112-591	1:100
CD16	3G8	BV650	Biolegend	302042	1:150
CD103	Ber- ACT8	BV785	Biolegend	350230	1:150
CD49a	TS2/7	FITC	Biolegend	328308	1:100
CCR7	G043H7	BV421	Biolegend	353208	1:100
CD69	FN50	PE	Biolegend	310906	1:100
CD62L	DREG- 56	AF700	Biolegend	304820	1:100

Eomes	WD1928	APC	Invitrogen	50-4877-42	1:100
Granzyme B	GB11	BV421	Biolegend	515408	1:100

Supplemental Table 1: Antibody panel used for cyclic immunofluorescence of FFPE pediatric tonsil sections

Antigen	Clone	Vendor	Catalog #	Conc (ug/mL)
Ki67	B56	Fluidigm	3172024B	5
CXCL12	79018	RnD	MAB350-SP	8
CD3	Polyclonal	abcam	AB5690	1.5
CD56	Polyclonal	RnD	AF2408	2.5
CXCR4	UMB2	abcam	ab124824	2
CD34	QBEnd/10	Novus	NBP2-34713	5

Supplemental Table 2: Quantification of migration parameters for migration plots shown and for additional biological donors.

CD56+ NK cells (Donor 1) + vehicle or AMD3100 on EL08.1D2 (Fig. 3C)							
metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	0.9857	10994	1.2336	27370	4.3209E-27
arrest coefficient	per track	fraction	0.4830	2271	0.3740	6204	1.6277E-32
aspect ratio	per frame	unitless	1.3185	10994	1.3123	27370	8.8468E-07
CD56+ NK cells (Donor 2) + vehicle or AMD3100 on EL08.1D2 (Supp. Fig. 4A)							
metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	1.0969	8123	1.2407	13363	5.2739E-04
arrest coefficient	per track	fraction	0.4227	856	0.3741	1715	4.2769E-04
aspect ratio	per frame	unitless	1.2456	8123	1.2873	13363	3.7699E-04
CD34+ HSPCs (Donor 1) + vehicle or AMD3100 on EL08.1D2 (Fig. 3D)							
metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	0.8266	7206	0.8816	22568	5.1072E-02
arrest coefficient	per track	fraction	0.5367	1245	0.5099	3944	1.7822E-02
aspect ratio	per frame	unitless	1.4078	7206	1.3139	22568	1.3626E-18
NK92 WT vs NK92 CXCR4 KO (Replicate 1) on EL08.1D2 (Supp. Fig. 4B)							
metric	measurement type (per frame or per track)	units	WT_mean	WT_N	KO_Mean	KO_N	p KO vs WT
speed	per frame	µm/min	1.6312	5267	1.6720	6757	4.7481E-01
arrest coefficient	per track	fraction	0.4322	48737	0.4039	73563	3.3858E-05
aspect ratio	per cell	unitless	1.2601	5267	1.2708	6757	5.3127E-03
NK92 WT vs NK92 CXCR4 KO (Replicate 2) on EL08.1D2 (Plots not shown)							
metric	measurement type (per frame or per track)	units	WT_mean	WT_N	KO_Mean	KO_N	p KO vs WT
speed	per frame	µm/min	1.2012	513889	1.5097	299843	0.0000
arrest coefficient	per track	fraction	0.5306	27973	0.4130	16413	4.8415E-50
aspect ratio	per cell	unitless	1.2194	513889	1.2409	299843	0.0000
CD56+ NK cells (Donor 1) + vehicle or AMD3100 on ICAM surface (Sup. Fig. 4C)							
metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	2.5538	25619	2.8919	26903	1.5578E-04
arrest coefficient	per track	fraction	0.5585	1322	0.5165	1165	1.0279E-03
aspect ratio	per frame	unitless	1.5907	25619	1.5782	26903	3.4494E-03
CD56+ NK cells (Donor 2) + vehicle or AMD3100 on ICAM surface (Plots not shown)							

metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	2.7770	54093	3.0310	46307	2.2940E-04
arrest coefficient	per track	fraction	0.2835	3268	0.2616	2409	7.5613E-03
aspect ratio	per frame	unitless	1.4740	54093	1.4959	46307	6.0714E-01
CD56+ NK cells (Donor 1) + vehicle or AMD3100 on VCAM surface (Supp. Fig. 4C)							
metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	3.1107	17919	3.1657	10507	2.2426E-05
arrest coefficient	per track	fraction	0.4863	1423	0.4055	698	2.8182E-09
aspect ratio	per frame	unitless	1.4312	17919	1.4815	10507	3.5592E-04
CD56+ NK cells (Donor 2) + vehicle or AMD3100 on VCAM surface (Plot not shown)							
metric	measurement type (per frame or per track)	units	Vehicle_mean	Vehicle_N	AMD_Mean	AMD_N	p AMD vs Vehicle
speed	per frame	µm/min	0.9367	51977	1.9258	16393	4.8629E-14
arrest coefficient	per track	fraction	0.6560	433	0.4175	154	8.1200E-14
aspect ratio	per frame	unitless	1.4502	51977	1.3194	16393	2.9630E-02
CD56+ NK cells from WHIM patient (Donor #7) after plerixafor or filgrastim treatment on EL08.1D2 (Fig. 6D)							
metric	measurement type (per frame or per track)	units	Filgrastim_mean	Filgrastim_N	Plerixafor_Mean	Plerixafor_N	p plerix vs filgr
speed	per frame	µm/min	0.6777	15444	0.8724	44817	5.0241E-09
arrest coefficient	per track	fraction	0.5125	2006	0.4489	7394	9.7301E-13
aspect ratio	per frame	unitless	1.2608	15444	1.1886	44817	7.5587E-63
CD56+ NK cells from WHIM patient (Donor #1) after plerixafor or filgrastim treatment on EL08.1D2 (Plots not shown)							
metric	measurement type (per frame or per track)	units	Filgrastim_mean	Filgrastim_N	Plerixafor_Mean	Plerixafor_N	p plerixafor vs filgrastim
speed	per frame	µm/min	0.5542	11295	0.5565	2307	3.8647E-04
arrest coefficient	per track	fraction	0.5689	1265	0.4818	189	7.8480E-03
aspect ratio	per frame	unitless	1.1959	11295	1.1183	2307	9.8265E-10